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Research report

Kinematic gait parameters are highly sensitive measures of motor deficits and spinal cord injury in mice subjected to experimental autoimmune encephalomyelitis

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HIGHLIGHTS

- Kinematic gait analyses identified movement deficits before the onset of clinical signs in mice subjected to experimental autoimmune encephalomyelitis (EAE).
- Gait deficits were observed in mice with mild EAE that failed to show clinical disease signs or altered rotarod performance.
- Impaired movement of the ankle correlated near perfectly with the degree of white matter loss detected in the spinal cord (r = 0.96).
- Kinematic gait analyses should assist the selection of promising therapeutic candidates for clinical testing.

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ABSTRACT

The preclinical selection of therapeutic candidates for progressive multiple sclerosis (MS) would be aided by the development of sensitive behavioural measures that accurately reflect the impact of autoimmune-mediated spinal cord damage on locomotion. Neurological deficits in mice subjected to experimental autoimmune encephalomyelitis (EAE) are typically scored using a clinical scale with 5–10 levels of increased disease severity. This ordinal scale represents a general impression of paralysis and impaired gait. By contrast, kinematic gait analyses generate ratio level data that have frequently been used to characterize walking deficits for MS patients and test the efficacy of treatments designed to improve them. Despite these advantages, kinematic gait analyses have not been systematically applied to the study of walking deficits for EAE mice. We have therefore used high speed video recordings (250 frames/s) of EAE mice walking on a treadmill to measure 8 kinematic parameters in the sagittal plane: average hip height (1), average toe height during swing (2), and average angle and range of motion for the hip (3–4), knee (5–6) and ankle (7–8). Kinematic measures of hip, knee and ankle movements were found to be early detectors of impaired locomotion for mice with mild EAE (median clinical score = 1.0 at day post-immunization 26; DPI 26). These deficits occurred in the absence of reduced rotarod performance with impaired hip and knee movements observed 3 days before disease onset as determined by clinical scores. Gait deficits for mild EAE mice were minor and often recovered fully by DPI 30. By contrast, severe EAE mice (median clinical score = 2.5 at DPI 26) displayed much larger movement impairments for the knee and ankle that failed to completely recover by DPI 44. Moreover, impaired ankle movement was highly correlated with white matter loss in the spinal cords of EAE mice (r = 0.96). Kinematic analyses therefore yield highly sensitive measures of motor deficits that predict spinal cord

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injury in EAE mice. These behavioural techniques should assist the selection of promising therapeutic candidates for clinical testing in progressive MS.

1. Introduction

Experimental autoimmune encephalomyelitis (EAE) models autoimmune mechanisms that trigger inflammatory demyelination and axonal damage in the spinal cord responsible for paralysis characteristic of multiple sclerosis (MS) [1–6]. Although current MS therapeutics effectively reduce disease exacerbations in relapsing-remitting MS, these treatments do not halt the neurodegenerative processes that drive permanent motor deficits in progressive MS [7,8]. The development of therapeutics that target these neurodegenerative events may be enabled by the use of sensitive behavioural tests that accurately predict the negative impact of spinal cord damage on locomotion for EAE mice [9]. Ideally, these behavioural assays should have sufficient sensitivity and power to detect motor impairments in the early stages of EAE using a minimum number of experimental animals. In these ways, such experimental techniques would allow promising therapeutic candidates to be identified so that the 3Rs of ethical policy in animal research (replacement, reduction and refinement) are better satisfied [10].

According to PubMed, over 11,000 publications have used a clinical scale, with 5 levels of increased neurological impairment, to rank disease severity and impaired movement in EAE mice [11]. In an attempt to improve the accuracy of clinical scoring, we have developed a more comprehensive scale with 10 levels of increasing EAE disease severity [12]. Clinical scoring for EAE is similar to the use of the EDSS to rate clinical disabilities in MS. EDSS encompasses 10 levels of increased clinical disabilities with moderate to high scores biased towards mobility deficits – 4 represents the onset of significant gait deficits, 6 reflects an inability to walk without assistance and 7 indicates that a subject is wheelchair-bound [13]. However, both clinical scoring for EAE and the EDSS for MS yield only ordinal data that provide little information about the degree and nature of motor deficits [13]. Furthermore, differences in the manner in which clinical scoring is conducted by various laboratories may contribute to variations in experimental findings obtained with EAE models [14]. The rapid elevation of clinical scores to levels that reflect irreversible axonal injury ($\geq$2.5) [4] also makes it difficult to test the efficacy of therapeutic interventions given at early stages of EAE. These limitations emphasize the need for improved measures of motor deficits resulting from EAE.

Preclinical testing has identified at least nine distinct classes of FDA-approved drugs that may be repurposed for the treatment of progressive MS [15–23]. One of these drugs classes, represented by pioglitazone and gemfibrozil, prescribed for the treatment type II diabetes [24], appears to enhance motor recovery for EAE mice by promoting the resolution of neuroinflammation, and increasing remyelination, in the spinal cord [18,25–29]. Moreover, three small clinical trials have shown that pioglitazone reduced relapses, decreases lesion burden and improves myelin integrity in patients with relapsing-remitting MS [30–32]. Since phase II/III trials designed to test these clinical endpoints are expensive, complicated and prolonged, it is crucial that the best therapeutic candidates be selected for human testing. We propose that this selection process would be aided by the development of behavioural tests that accurately reflect the impact of spinal cord injury on movement. The recent development of innovative platforms that enable small molecules, which stimulate oligodendrogenesis and promote remyelination, to be rapidly identified in large compound libraries [19,21,22] will likely increase this demand.

Approximately 75% of MS patients suffer from walking deficits [33,34]. Kinematic gait analyses have been used to describe how specific aspects of joint movement change for MS patients during walking [35,36]. Our PubMed searches have identified over 125 clinical studies that have employed gait analyses to study walking deficits in MS [36] and the efficacy of therapeutic interventions designed to improve them (drugs, orthotic braces, stretching, exercise) [35,37]. However, the use of kinematic gait analyses to assess motor deficits in EAE has only been reported once [38]. As a result, we have employed kinematic gait analyses to examine how walking is altered for mice during the acute and chronic phases of MOG_{35-33}-induced EAE. Kinematic measures of hip, knee and ankle movements during a step cycle were compared with clinical scoring and rotarod performance to determine the behavioural tests that best correlated with the degree of white matter loss detected in the spinal cords of EAE mice. Our findings indicate that these kinematic measures offer improved sensitivity and accuracy in the detection of both early and persistent motor deficits resulting from autoimmune-mediated spinal cord injury.

2. Materials and methods

2.1. Animal care

All experiments were done in accordance with the ARRIVE and Canadian Council on Animal Care guidelines and were approved by the Dalhousie University Committee on Laboratory Animals. Mice were housed in the Life Science Research Institute Animal Care Facility on a 12-h light/dark cycle (light from 07:00 to 19:00). Food and water were provided ad libitum.

2.2. Experimental autoimmune encephalomyelitis

C57BL/6 mice were obtained from Charles River Canada (St. Constant, QC) and were allowed to habituate to the facility for seven days before experiments commenced. Ten week-old female mice were either immunized with amino acids 35–55 (MEVGWYRSPFSRVVHLYRNGK) of myelin oligodendrocyte glycoprotein (MOG_{35-55}) to induce EAE, or were given a sham immunization with complete Freund's adjuvant (CFA). CFA mice did not develop EAE and served as antigen controls. The average weight and rotarod performance prior to immunization were the same for the CFA and EAE groups. To induce EAE, MOG_{35-55} (Gen Script, Piscataway, NJ, USA) was dissolved in sterile phosphate buffered saline (PBS; pH = 7.4) at a concentration of 3 mg/ml and emulsified in a 1:1 ratio with complete Freund's adjuvant (CFA). CFA was made by mixing incomplete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA) with heat-killed Mycobacterium tuberculosis H37RA (Difco Laboratories, Detroit, MI, USA) at a 10 mg/ml concentration. The MOG_{35-55}/CFA emulsion was delivered via two subcutaneous (sc) injections (100 μl/injection) on both sides of the base of the tail so that each mouse received a total of 300 μg of MOG_{35-55}. For the CFA group, PBS and CFA were emulsified at a 1:1 ratio and injected in the same manner as for EAE mice. All mice were injected with pertussis toxin (PTX; Sigma, St. Louis, MO, USA) on day 0 and 2